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Evaluation of Bacterial Diversity Dynamics During the Composting of Microbial Fertilizer with Inoculant Addition

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Abstract

In recent years, microbial biofertilizer has been widely applied as a crucial tool for enhancing soil fertility, promoting plant growth, and increasing crop yields and plays an increasingly prominent role in sustainable agricultural practices. The microbial communities in microbial biofertilizers modulate the efficiency of the composting process and the quality of the final product via diverse ecological functions, including organic matter degradation, nitrogen cycling, and carbon fixation. However, the dynamic shifts in microbial communities during composting and their intricate interactions with environmental factors remain insufficiently investigated. This study aimed to assess the dynamic shifts and diversity profiles of bacterial communities during the composting of microbial biofertilizers. The abundance, diversity, and community structural dynamics of bacteria across distinct composting stages were analyzed using PCR-DGGE and Illumina MiSeq sequencing technologies. The compositional variations in bacterial communities across different composting stages were compared using alpha diversity indices (e.g., Chao1, Shannon, Simpson), beta diversity analysis, and principal coordinate analysis (PCoA). The composting feedstocks included cow manure, straw, and a liquid inoculant of Bacillus sphaericus thermophilus (a thermophilic spherical urea-degrading bacillus). These materials were mixed at a specific ratio and subjected to aerobic composting. Samples were collected on days 10, 20, 30, 40, and 50 of composting (designated groups S1 to S5) to characterize the temporal succession dynamics of the microbial communities. The results demonstrated that the number of bacterial operational taxonomic units (OTUs) changed significantly over the composting period: the S1 group exhibited the highest OTU richness, while the S5 group had the lowest. Additionally, across different composting stages, the phylum Firmicutes dominated the initial phase but gradually decreased in relative abundance, whereas the abundance of phyla such as Proteobacteria progressively increased in the later stages. Taxonomic analysis revealed that Ureibacillus thermophilus was relatively abundant during the early composting stage, whereas the abundance of Sporosarcina and Sphaerobacter peaked during the middle stage. In contrast, the abundance of genera such as Taibaiella increased during the late composting stage. Principal coordinate analysis (PCoA) further revealed that bacterial communities differed significantly between samples from distinct composting stages. Microbial co-occurrence network analysis revealed that the microbial community exhibited small-world and modular properties during composting. The 17 identified keystone genera were primarily affiliated with five phyla, including Proteobacteria. Despite their low relative abundances, these keystone genera may play potentially critical roles in mediating the composting process. This study offers novel insights into the succession of microbial communities during composting and further elucidates the associations between shifts in microbial community diversity, composting efficiency, and the quality of the final compost product. The findings of this study provide a theoretical foundation for optimizing composting processes and enhancing composting efficiency while also offering valuable insights for the application and development of microbial inoculants. Future research may further investigate the specific contributions of distinct functional microbial taxa to the composting process, as well as strategies for optimizing microbial community assembly via environmental modulation to enhance composting performance and end-product quality.

1. Introduction

As a critical strategy for the resource recovery of organic waste, composting is highly important for advancing sustainable agricultural development and ecological environmental protection(Sequi 1996). The composting process inherently occurs in complex ecosystems, where microorganisms drive the stabilization and transformation of waste via organic matter degradation, nutrient cycling, and carbon sequestration(Xu et al. 2024). Environmental factors, including nutrient availability, temperature, pH, moisture content, and oxygen level, dynamically change throughout the composting process, profoundly modulating the composition and functional traits of the microbial community(Mao et al. 2020). Concurrently, the structure of the microbial community continuously evolves as organic matter decomposes, a process that has a deterministic influence on composting efficiency and the quality of the end product(Zhang et al. 2024). Thus, an in-depth dissection of the succession dynamics of microbial communities—particularly their functional roles in organic matter decomposition—can provide a critical theoretical foundation for the optimization of composting technologies.

In recent years, with the advancement of composting technologies, a growing body of research has focused on the functional roles of microbial communities throughout the composting process. Notably, during the composting process, specific microbial genera play pivotal functional roles. For example, during the initial phase of composting, thermophilic taxa such as Ureibacillus thermophilus and Bacillus dominate the rapid degradation of organic matter, whereas in the later phase, phyla such as Proteobacteria and Bacteroidetes facilitate the further transformation of organic matter and nitrogen cycling. Furthermore, as the composting process progresses, the diversity of the microbial community markedly shifts, resulting in a deterministic influence on both the composting efficiency and the quality of the end product(Zhang et al. 2024; Malal et al. 2025; Papale et al. 2021; Zhang et al. 2025; Zhou et al. 2024; Du et al. 2025). Changes in the function of microbial communities have a pivotal effect on the efficiency of composting. During the composting process, the microbial community gradually undergoes functional specialization, with core functions encompassing organic matter degradation, nitrogen cycling, and carbon fixation. Notably, the interaction between nitrogen-fixing bacteria and nitrifying bacteria enhances nitrogen use efficiency during the composting process(Zhong et al. 2020; Hernández-Lara et al. 2022; Wang et al. 2014; Liu et al. 2025; Chen et al. 2024; Chang & Hsu 2008; Said-Pullicino et al. 2007; Dan et al. 2017). Concurrently, the interspecific interactions within the microbial communities of the compost and their network structures also play a pivotal role in the succession process. Microbial co-occurrence network analysis can reveal the synergistic relationships among distinct microbial taxa and their regulatory effects on composting dynamics(Duan et al. 2023).

In this study, PCR-DGGE and Illumina MiSeq sequencing technologies were employed to conduct an in-depth analysis of the abundance, diversity, and structural dynamics of microbial communities during composting, with a specific focus on the temporal succession of microbial communities across distinct composting stages(Wang et al. 2022; Chen et al. 2022). Here, beta diversity analysis, principal coordinate analysis (PCoA), and microbial co-occurrence network analysis were employed to explore the succession patterns of microbial communities during composting and their associations with

environmental factors. Two research questions are addressed herein: What is the association between shifts in microbial community diversity and key environmental factors (e.g., temperature, humidity, and pH) during composting? Which key microbial genera act as core drivers during composting, and how do their functional traits regulate composting efficiency? Addressing these questions will facilitate a comprehensive understanding of the succession dynamics of microbial communities during composting and their associations with environmental factors, thereby providing novel theoretical insights for the optimization of composting processes and the development of microbial inoculants. Furthermore, this study employed network analysis to investigate interspecies interactions and functional divergence within microbial communities during composting, thereby providing a foundation for enhancing composting efficiency and product quality.

2. Materials and Methods

2.1 Experimental reagents

(1) Polyvinylpyrrolidone (PVP), phenylalanine, bovine serum albumin, Coomassie Brilliant Blue G-250, anthrone, 3,5-dinitrosalicylic acid (DNS), and crystalline phenol were all of analytical grade and were purchased from Tianjin Damo Chemical Reagent Factory.

(2) The OMEGA kit and Qubit 3.0 DNA Assay Kit were obtained from Shanghai Sangon Biotechnology Co., Ltd.

2.2 Preparation of Microbial Inoculants

2.2.1 Raw Materials and Formulation Ratios for Inoculant Preparation

Cow dung (1000 kg), straw (25 kg; corn straw), and a bacterial suspension of Ureibacillus thermosphaericus (7.5 kg) were used as the primary raw materials.

The straw was first comminuted using a pulverizer, with the particle size controlled to be ≤ 2 mm. The comminuted straw was mixed with cow dung at the specified ratio, after which Ureibacillus thermosphaericus bacterial suspension and water (2.5 kg) were added to adjust the initial moisture content of the mixture to 60 ± 1%. This mixture was subsequently employed as the raw material for composting.

2.2.2 Compost formulation ratios and operating conditions

The base diameter and height of the compost pile (formed from the mixture) were 145 cm and 95 cm, respectively, and the internal moisture content was maintained between 60 and 65%. The mixture was subjected to manual turning: once every 3 days during the heating and thermophilic phases and once every 7 days during the cooling phase. The composting temperature was maintained within the range of 50–70°C to ensure microbial activity.

Sample Collection: During the composting process, samples were collected on days 10, 20, 30, 40, and 50, which were designated Groups S1, S2, S3, S4, and S5, respectively. For each sample group, sampling was conducted at a depth of 40 cm below the pile surface. Three replicate samples were collected per group and then homogenized to form a composite sample, ensuring sample uniformity.

2.2.3 Preparation and Cultivation of Bacterial Suspensions

During the bacterial suspension preparation process, a mixture consisting of 1000 kg of cow manure and 25 kg of straw was used, followed by the addition of 7.5 kg of Ureibacillus thermosphaericus bacterial suspension. The culture medium was fermented at 30–35°C, with an aeration rate of 100 L/h and a rotation speed controlled at 200 r/min. Fermentation was conducted for 72 hours, yielding a high-concentration bacterial suspension.

2.2.4 Composting Treatment and Environmental Parameter Control

During the composting process, the environmental conditions of the compost pile were regulated by controlling its moisture content, temperature, and oxygen level to ensure an optimal growth environment for the microorganisms. During the composting process, the temperature of the compost pile was maintained within the range of 50–70°C to ensure the metabolic activity of the thermophilic microorganisms. During the composting process, the compost pile was turned at regular intervals to ensure uniform decomposition of organic waste and adequate aeration.

2.2.5 Post-Composting Processing and Quality Control

After a specified composting period, the maturity and stability of the microbial inoculant fertilizer meet the corresponding technical standards. All the compost samples were processed at distinct time points; subsequently, biochemical and molecular analyses were performed on the samples to verify the activity and efficacy of the microbial inoculant. Postprocessed compost can be applied in agricultural settings to further improve soil fertility.

2.3 Sample Collection and Preparation Methods

2.3.1 Compost Sampling and Preparation

Microbial inoculant fertilizer was produced via aerobic composting using cow manure, crop straw, and a bacterial suspension of Ureibacillus thermosphaericus (a thermophilic spherical urea-degrading bacillus). The base diameter of the compost pile was 145 cm, and the height was 95 cm, with the moisture content maintained between 60 and 65%. The temperature and moisture content of the compost pile were maintained within the optimal range through environmental factor regulation to ensure microbial activity and composting efficiency.

2.3.2 Sample Collection

The compost samples were collected on the 10th, 20th, 30th, 40th, and 50th days of the composting process and were designated groups S1, S2, S3, S4, and S5, respectively. For each composting stage, samples were collected from 40 cm below the pile surface; three subsamples were taken from three distinct sampling points for each sample group to ensure the representativeness and homogeneity of the composite samples. All the subsamples were pooled into a composite sample immediately after collection, which was subsequently used for subsequent microbial analyses.

3. Research Methods

2.1 Extraction of Total Microbial Genomic DNA

A total of 0.1 g of freshly collected microbial biofertilizer was weighed, and total DNA was extracted using the E.Z.N.A.® Stool DNA Kit following the manufacturer's protocol. The integrity of the extracted DNA was assessed via 2% agarose gel electrophoresis.

2.2 Gene amplification and high-throughput sequencing

The extracted genomic DNA was subsequently used for 16S rRNA gene library preparation and high-throughput sequencing. This process comprises four sequential steps: first-round PCR amplification, second-round PCR amplification, PCR product purification, and sequencing on the instrument.

Prior to high-throughput sequencing, three DNA aliquots extracted from each soil sample were thoroughly pooled and used as the template for first-round PCR amplification. Genomic DNA was precisely quantified using a detection kit to determine the optimal volume of DNA template to be added to the PCR. The PCR primers employed were fused with the V3-V4 region universal primers compatible with the MiSeq sequencing platform: 341F primer (5'-CCTACGGGNGGCWGCAG-3') and 805R primer (5'-GACTACHVGGGTATCTAATCC-3'). The PCR system was composed of 12.5 μL of 2× Master Mix, 2.5 μL of forward primer, 2.5 μL of reverse primer, 50 ng of template DNA, and ddH_2O such that the total volume was 25 μL. The PCR amplification conditions were as follows: predenaturation at 98°C for 30 s; 5 cycles of denaturation at 98°C for 10 s, annealing at 54°C for 30 s, and extension at 72°C for 45 s; 15 cycles of denaturation at 72°C for 10 min, annealing at 54°C for 30 s, and extension at 72°C for 45 s; and a final extension at 72°C for 10 min; and storage at 4°C.

The second round of PCR amplification was performed to incorporate Illumina bridge PCR-compatible primers, thereby enabling Illumina sequencing. The second-round PCR system was composed of 12.5 μL of 2× Master Mix, 2.5 μL of forward primer, 2.5 μL of reverse primer, 50 ng of template DNA, and ddH_2O such that the total volume was 25 μL. The prepared PCR mixture was subjected to amplification under the following thermal cycling conditions: predenaturation at 98°C for 30 s; 5 cycles of denaturation at 98°C for 10 s, annealing at 54°C for 30 s, and extension at 72°C for 45 s; and a final extension at 72°C for 10 min; and storage at 4°C.

PCR product purification and quantification: Following completion of PCR amplification, the resulting PCR products were purified and recovered using AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA). The purified PCR products were subsequently subjected to 2% agarose gel electrophoresis via Pippin Prep (DNA Size Selection for Next-Generation Sequencing), followed by the collection of fragments with an approximate size range of 250–350 bp. The fragment size distribution was subsequently validated using a TapeStation system (Agilent 4200). Finally, the recovered fragments were precisely quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) with its corresponding assay kit. All the purified amplification products from the samples were pooled at equimolar concentrations: 10 ng of DNA was aliquoted from each sample, resulting in a final sequencing loading concentration of 20 pmol. The amplicon pool was subjected to sequencing. The size and quantity of the amplicon library were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and an Illumina-compatible library quantification kit (Kapa Biosciences, Woburn, MA, USA), respectively. Sequencing was performed on the NovaSeq PE250 platform.

3.3 Bioinformatics Analysis

Five sample groups were sequenced on the Illumina NovaSeq platform, with sequencing services provided by LC-Bio (LC Sciences, Hangzhou, China). Paired-end sequences were demultiplexed to their respective samples on the basis of unique sample-specific barcodes, followed by the removal of library construction-introduced barcodes and primer sequences. Paired-end reads were merged using fast length adjustment of short reads (FLASH). Raw read data were subjected to quality filtering under predefined criteria using fqtrim (v0.94) to generate high-quality clean tags. Chimeric sequences were filtered using Vsearch (v2.3.4). DADA2 was employed for sequence denoising to generate the feature table and feature sequences. Alpha and beta diversities were calculated following normalization to the same number of random sequences (rarefaction). Subsequently, feature abundances were normalized by scaling to the relative abundance of each sample, with taxonomic classification performed using the SILVA classifier (release 132).

Alpha diversity was used to characterize the complexity of intrasample species diversity, with five metrics (Chao1, ACE, coverage, Shannon, and Simpson) calculated using QIIME 2. Beta diversity was computed using QIIME 2, and the corresponding visualization was generated with an R package. Sequence alignment was performed using BLAST, and each representative sequence was taxonomically annotated against the SILVA database. All remaining visualizations were generated using R (version 3.5.2).

3.4 Statistical Analysis

All the experiments were performed with three biological replicates. The data are presented as the mean ± standard deviation. One-way analysis of variance (ANOVA) was conducted using SPSS 26.0 software, followed by Duncan's new multiple range test to assess intergroup differences ($p < 0.05$).

4. Results

4.1 Differences in Operational Taxonomic Unit (OTU) Profiles of the Microbial Community During Microbial Fertilizer Composting

The results of the alpha diversity analysis revealed no significant differences in the Ace and Chao1 indices across groups S1, S2, S3, and S4, with a relatively stable trend observed. These findings indicate that the sequencing results are highly reliable (Figure 1). The coverage index exceeded 0.94 across all the samples, demonstrating that the sequencing depth was sufficient to accurately characterize the structure of the bacterial community. In terms of the Ace and Chao1 indices, the S5 group presented significantly greater values than the other groups did ($P < 0.05$), indicating greater species richness of the bacterial community in the S5 group. The Shannon index was the highest in the S5 group samples, with significant differences observed compared with the other groups; the Simpson index was the lowest in the S1 group, and similarly, significant differences were detected between the S1 group and the other groups. Integrating the Ace, Chao1, and Shannon indices, the microbial fertilizer samples from group S5 demonstrated significantly greater bacterial community diversity.

The composition and dynamics of operational taxonomic units (OTUs) in samples collected at different time points during the composting of microbial fertilizer are as follows: Group S1 contained 166 unique OTUs, predominantly belonging to Firmicutes (65.06%), Proteobacteria (9.04%), and Bacteroidetes (8.43%). Group S2 contained 149 unique OTUs, with notable shifts in the proportions of Firmicutes (38.26%), Proteobacteria (10.07%), and Bacteroidetes (12.75%). Group S3 contained 123 unique OTUs, with further shifts in the proportions of Firmicutes (20.33%), Proteobacteria (34.96%), and Bacteroidetes (12.2%) (Figure 2).

During the late stage of composting, the number of unique OTUs in Group S4 increased to 249, with significant increases observed in the proportions of Firmicutes (13.25%), Proteobacteria (26.1%), Actinobacteria (13.25%), Acidobacteria (9.24%), and Gemmatimonadetes (7.23%). Group S5 contained 1042 unique OTUs, with a significant reduction in the proportion of Firmicutes (13.98%) and significant increases in those of Proteobacteria (33.37%), Planctomycetes (10.02%), Bacteroidetes (9.52%), and Actinobacteria (5.91%).

These results indicate that the operational taxonomic unit (OTU) composition of the microbial community significantly shifted during composting. Firmicutes dominated the early composting stage, whereas taxa such as Proteobacteria and Planctomycetes became dominant in the late stage, reflecting a gradual increase in the diversity and complexity of the microbial community throughout the composting process.

4.2 Taxonomic Classification Analysis

The naïve Bayesian assignment algorithm was employed to analyze each sequence, with matching probability values calculated across different taxonomic ranks to determine the taxonomic classification corresponding to each OTU. Typically, a classification result is considered reliable if the matching probability value exceeds 0.8. A total of 27,455 OTUs were obtained from the analysis of the five sample

groups. Following the taxonomic classification of these OTUs, the results were assigned to 49 phylogenetic groups alongside a number of unclassified bacterial taxa. The dominant taxonomic groups included *Firmicutes, Proteobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Acidobacteria, Verrucomicrobia*, and *Deinococcus-Thermus* (Figure 3). Notably, *Firmicutes* and *Proteobacteria* exhibited the highest abundances across all the samples, accounting for 1.81–94.46% and 2.22–37.83% of the total sequences, respectively. In the bacterial communities of the five microbial fertilizer compost groups, the relative abundance of *Firmicutes* decreased significantly with increasing duration of composting, with an extremely significant difference observed between groups S1 and S5 ($P < 0.01$). In the bacterial communities of groups S1, S2, and S3, *Firmicutes*, *Proteobacteria*, and *Chloroflexi* were the dominant phyla; in contrast, the dominant phyla in groups S4 and S5 were *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Actinobacteria*.

The distribution patterns of bacterial genera differed significantly across the distinct sample groups. Specifically, in the group S1 samples, *Ureibacillus, Lysinibacillus, Symbiobacterium, Tepidimicrobium*, and *Ruminiclostridium* exhibited relatively high relative abundances; in group S2, *Sporosarcina, Sphaerobacter, Vulgatibacter, Trueperella*, and *Ureibacillus* were more abundant; in group S3, *Sporosarcina, Sphaerobacter, Ruminococcaceae_UCG-012, Vulgatibacter, Trueperella*, and *Paenibacillus were predominant*; in group S4, *Ruminofilibacter, Pseudomonas*, and *Chryseolinea* were relatively abundant; and in group S5, *Taibaiella, Roseiflexus, Thermomonospora, Chryseolinea, H16*, and *Steroidobacter* were relatively abundant.

The relative abundance of Ureibacillus thermophilus was greatest during the initial stage of composting, and its relative abundance gradually decreased with increasing duration of composting. The relative abundance of Sporosarcina and Sphaerobacter was greatest in the mesophilic/middle stage of composting, whereas *Taibaiella*, *Roseiflexus*, and *Thermomonospora* accumulated in the thermophilic/maturation stage and reached their peak relative abundances. Microbial inoculant fertilizer enhances fertilizer quality through the addition of Ureibacillus thermophilus. During different composting stages, the abundance of the genus Ureibacillus remained stable among the top 50 genera, indicating that Ureibacillus plays a sustained functional role throughout the composting process. Furthermore, the relative abundance of the genus Ureibacillus initially decreased but subsequently increased, with the abundance in stage S5 being greater than that in stage S4. This phenomenon may be associated with the stability of environmental conditions during the late composting period.

4.3 Beta Diversity Analysis and Statistical Analysis of Intergroup Differences

In this study, principal coordinate analysis (PCoA) was used to compare the overall variations in bacterial community composition among the five sample groups (Figure 5). On the basis of the relative abundance of operational taxonomic units (OTUs) derived from the bacterial 16S rRNA gene, samples from groups S2 and S3 clustered together, whereas those from groups S4 and S5 exhibited relatively similar distribution patterns. Group S1 was distinctly separated from the other four groups by a greater distance. The principal coordinates PC1 and PC2 accounted for 34.37% and 31.52% of the total variation, respectively, and were effective at discerning the similarities and dissimilarities among samples. With

respect to the three replicate samples, overall, the composition of the bacterial community changed significantly, with distinct differences observed among the bacterial communities in the early, middle, and late stages of composting. e group were closely clustered, indicating high consistency among biological replicates.

Furthermore, three complementary nonparametric multivariate statistical tests (Adonis, ANOSIM, and MRPP) further confirmed the consistency of the results across the three analytical approaches. No significant difference was detected in the bacterial communities between groups S1 and S2. In contrast, significant differences were observed between groups S2 and S3 ($P < 0.05$), S3 and S4 ($P < 0.05$), and S4 and S5 ($P < 0.05$) (Table 1). This study further confirmed that significant differences existed in the bacterial communities across different composting stages, with the exception of the S1 and S2 groups during the initial composting phase.

Table 1. Significance tests of the effects on the bacterial community structure with three different statistical approaches

	Adonis		ANOSIM		MRPP	
	F	*P*	*R*	*P*	*δ*	*P*
total	81.592	**0.001**	0.9778	**0.001**	0.1076	**0.001**
S1:S2	52.384	0.1	1	0.1	0.07757	0.1
S2:S3	44.398	0.003	0.9424	0.005	0.09254	0.008
S3:S4	78.752	0.001	0.963	0.001	0.1041	0.001
S4:S5	81.592	0.001	0.9778	0.001	0.1076	0.001

4.4 Community Network Analysis of Microbial Inoculant Fertilizers

Using the operational taxonomic unit (OTU) data generated via high-throughput sequencing, a microbial molecular ecological network was constructed for the composting process of microbial inoculant fertilizer, and the topological properties of its network structure were analyzed. Following data preprocessing, a phylogenetic molecular ecological network comprising 381 operational taxonomic units (OTUs) was generated (Table 2). Both the average path length and the average clustering coefficient of the network were significantly greater than those of random networks, indicating that the network exhibits small-world properties, where most nodes can be interconnected via a relatively small number of steps or hops. Further analysis revealed that the modularity value of the network was significantly greater than that of random networks, indicating that the network exhibited modular characteristics. Overall, compared with random networks, the phylogenetic molecular ecological network contained a greater number of highly connected nodes, and its topological property values were also significantly greater than those of random networks, indicating that this network is a scale-free nonrandom network.

Table 2. Topological properties of molecular ecological networks (MENs) of different microbial communities and their associated random MENs

Network structure	Topological Properties	S1
Phylogenetic Molecular Ecological Network	Similarity Threshold	0.87
	Total Number of Nodes	381
	Total Number of Edges	1687
	Average Degree	8.870
	Betweenness Centrality	0.120
	Degree Centrality	0.106
	Average Path Length	8.856
	Average Clustering Coefficient	0.183
	Modularity	0.488
Random Network	Modularity	0.277±0.004
	Average Path Length	3/015±0.017
	Average Clustering Coefficient	0.063±0.006

In this study, pairwise correlations of operational taxonomic unit (OTU) relative abundances across different samples were employed to define the adjacency matrix and facilitate network construction. On the basis of this adjacency matrix, a network graph was constructed to characterize the positive or negative interactions between different operational taxonomic units (OTUs) (Figure 6). In this ecological network, Firmicutes was the most represented phylum, accounting for 40.68% of the nodes, with all the OTUs within this phylum showing positive correlations. Further analysis revealed that the relative abundances of phyla, including Firmicutes, Chloroflexi, Gemmatimonadetes, Acidobacteria, Hydrogenedentes, and Bacteroidetes, were lower than the average node connectivity threshold, suggesting that these phyla play potentially critical roles in the network.

At the node connectivity level of the ecological network, the left panel displays the relative abundances of the top 5 most abundant phyla, while network connectivity is driven primarily by interactions between Firmicutes and other phyla. Notably, Firmicutes (49.26%), Proteobacteria (16.27%), Actinobacteria (8.33%), Chloroflexi (6.97%), and Planctomycetes (4.92%) accounted for a relatively high proportion of nodes in the network (Figure 7).

This study subsequently identified potential keystone taxa in the network, which primarily included peripheral nodes, connectors, module hubs, and network hubs. From the topological perspective of the network, distinct nodes fulfill divergent biological roles within the ecological network. The majority

(95.54%) of operational taxonomic units (OTUs) were classified as peripheral nodes (Pi < 0.62, Zi < 2.5), with most of these nodes exhibiting intramodule connectivity. Among the peripheral nodes, 56.43% exhibited no intermodule connections (Pi = 0) (Figure 8). Notably, none of the most critical keystone taxa were identified among the 381 operational taxonomic units (OTUs) in this network, with the number of network hubs (Pi > 0.62, Zi > 2.5) being zero (Figure X, if applicable).

The Sankey diagram illustrates the keystone taxa and their taxonomic ranks across distinct network structures. A total of 17 keystone taxa (operational taxonomic units, OTUs) were detected within the network architecture, comprising 9 connectors and 8 module hubs. The 9 genera associated with these nodes included *norank_Gemmatimonadaceae, Dethiobacter, norank_Gemmatimonadetes, Syntrophomonas, norank_Caldilineaceae, Roseiflexus, norank_D8A-2, Constrictibacter,* and *Steroidobacter* (Figure 9). The eight genera associated with the module hubs included *norank_Anaerolineaceae, Bacillus, Tepidimicrobium, norank_MBA03, norank_M55-D21, norank_OM1_clade, unclassified_Clostridia,* and *Parviterribacter*. These genera were predominantly affiliated with five distinct bacterial phyla, namely, Proteobacteria (1.6%), Actinobacteria (0.84%), Firmicutes (0.55%), Gemmatimonadetes (0.38%), and Chloroflexi (0.36%). Notably, the relative abundances of most module hubs and connector nodes were relatively low (< 1%), which underscores their potential critical ecological roles within the microbial community.

5 Discussion

5.1 Relationships between the Dynamic Succession of Microbial Communities and Environmental Factors

The dynamic succession of microbial communities and their interactions with environmental factors during composting critically influence composting efficiency and the quality of the final product. According to previous studies, shifts in microbial communities during the composting process are closely associated with the dynamics of environmental factors(Meng et al. 2019). Studies have demonstrated that during the initial phase of composting, as organic matter undergoes rapid decomposition, the thermophilic stage is dominated by thermophilic microorganisms—with a notable increase in taxa such as the genus Ureibacillus. As the composting process progresses, the temperature gradually decreases, driving shifts in the microbial community structure—with a progressive increase in the number of taxa adapted to the newly established environmental conditions. Our findings indicate that *Ureibacillus thermophilus* is relatively abundant during the initial phase of composting; as composting progresses, its relative abundance gradually decreases—a phenomenon associated with the stabilization of temperature and environmental conditions during the later composting stage. During the mid-composting phase, the abundances of *Sporosarcina* and *Sphaerobacter* increased; during the late composting stage, the relative abundances of *Taibaiella, Roseiflexus*, and *Thermomonospora* significantly increased. These shifts are closely associated with the metabolic demands for the decomposition and transformation of organic matter throughout the composting process.

Furthermore, fluctuations in the number of operational taxonomic units (OTUs) throughout the composting process mirror dynamic shifts in microbial community diversity. Our study revealed that the number of OTUs was greatest in Group S1 and lowest in Group S5, with statistically significant differences across distinct stages. This suggests that the diversity of the microbial community has undergone significant temporal changes, which may be closely associated with shifts in environmental factors (e.g., temperature, humidity, and pH) throughout the composting process(Zhang et al. 2019). The relatively high OTU count observed at the initial composting stage likely reflects the rich microbial diversity inherent in the raw composting materials. As the composting process progresses, shifts in environmental conditions drive a gradual decline in the abundance of maladapted microbial taxa, whereas the diversity and abundance of taxa adapted to the novel environment either stabilize or increase(Luo et al. 2024; Chang et al. 2021). Notably, within the OTU composition of Group S1, the phylum Firmicutes accounted for a relatively high proportion. In contrast, the abundance of Firmicutes in Group S5 decreased significantly, while the number of unique OTUs belonging to other phyla (e.g., Proteobacteria) increased. This suggests that as composting progresses, the structure of the microbial community markedly shifts, with a close interrelationship existing between microbial community succession and the dynamics of the composting environment. The phylum Firmicutes dominated the microbial community during the initial composting stage, and this taxon plays a crucial role in the decomposition of complex organic substrates (e.g., cellulose and lignin)(Wang et al. 2024; Zhong et al. 2020). However, as complex organic substrates were gradually depleted and small-molecule metabolites accumulated, the abundances of taxa such as the phyla Proteobacteria and Bacteroidetes increased significantly. These observations suggest that these taxa play critical roles in the subsequent transformation of organic matter and nutrient cycling(Gao et al. 2024; Prescott & Vesterdal 2021). The dominant status of the phylum Firmicutes was gradually displaced by that of other bacterial phyla, reflecting the adaptive succession of the microbial community in response to dynamic changes in the composting environment(Zhong et al. 2020).

Notably, the microbial community diversity tended to increase during the middle and late composting stages, with the bacterial community structure becoming more stable and its functional differentiation becoming more distinct. During the thermophilic phase, the compost was dominated by thermophilic microorganisms, whereas during the mesophilic/cooling phase, microbial diversity recovered and tended to stabilize(Ding et al. 2020). The relative abundance of the genus *Ureibacillus* was relatively high during the early composting stage. As composting progressed, its relative abundance gradually decreased, which may be associated with more stable environmental conditions during the late composting stage, thereby facilitating the growth and proliferation of other microorganisms(Liu et al. 2022). This phenomenon indicates that the succession of microbial communities during composting not only manifests as shifts in population composition but also reflects the functional transformation requirements of the community(Liu et al. 2020). These dynamic shifts indicate that microbial communities are capable of self-regulation in response to fluctuations in environmental conditions during composting, thereby allowing them to adapt to new ecological niches.

5.2 Role of Microbial Functions in Composting

During the composting process, the functions of microbial communities gradually become specialized and include organic matter degradation, nitrogen cycling, and carbon fixation, among others. Through taxonomic analysis of bacterial communities across different composting stages, we observed that in the early composting stage, microorganisms belonging to the phyla Firmicutes and Proteobacteria were primarily involved in the degradation of cellulose and lignin—which is consistent with previous findings regarding organic matter degradation processes during the initial composting phase(Wang et al. 2022). Notably, within the phylum Actinobacteria, certain species are known to exhibit efficient cellulose-degrading capabilities—which is consistent with the findings of our study(Hu et al. 2021). During the composting process, the thermophilic spore-forming genus Ureibacillus dominated the initial stage; its activity under high-temperature conditions likely contributed significantly to the rapid decomposition of complex organic matter and heat release(Wang et al. 2024; Cai et al. 2024). These observations suggest that thermophilic microorganisms perform critical ecological functions during the initial phase of composting.

As the composting process progresses, the functions of the microbial community gradually specialize, with an increasing number of bacterial taxa engaging in the further degradation of complex organic matter. In the late phase of composting, the abundances of Taibaiella and Roseiflexus increased significantly, suggesting that these taxa play a central role in organic matter metabolism and carbon–nitrogen cycling(Hoang et al. 2022). Furthermore, in the late phase of composting, Thermomonospora and Steroidobacter are involved in cellulose degradation and lipid metabolism, respectively, thereby further enhancing compost maturity and quality(Liao et al. 2023). These findings demonstrate that microbial functions during composting not only gradually specialize over time but also that distinct microbial communities play specific roles in the decomposition and transformation of organic matter.

5.3 Diversity of Microbial Communities and Composting Efficiency

The diversity of microbial communities directly influences the efficiency of the composting process and the quality of the final compost product(Wang et al. 2023). Analysis of the alpha diversity index revealed that the species richness and evenness of the samples in Group S5 were significantly greater than those in the other groups. These observations suggest that microbial community diversity gradually increases with increasing duration of composting, thereby optimizing composting performance(Yin et al. 2025). This observation aligns with the established relationship between microbial community diversity and composting performance reported in the literature. During the initial phase of composting, the microbial community is relatively simple and dominated by a limited number of microbial taxa that undergo preliminary degradation of organic matter. As the composting process progresses, the microbial community gradually diversifies, with the progressive colonization of novel microbial species; their synergistic interactions accelerate the transformation of organic waste and increase the stability and quality of the compost(Gao et al. 2024). Thus, the dynamic succession and diversity of microbial communities are critical to material cycling and energy flow throughout the composting process.

5.4 Analysis of Microbial Community Network Structure

Microbial molecular ecological network analysis revealed that the composting microbial community exhibited small-world properties, modular features, and a scale-free, nonrandom network structure. These findings align with previous research on the network structure of composting microbial communities. During the composting process, Firmicutes accounted for a relatively high proportion of the network, with positive correlations observed among its operational taxonomic units (OTUs); this suggests that Firmicutes may serve as a core taxon in mediating interactions within the composting microbial community(Zhu et al. 2021). Notably, across distinct stages of composting, Firmicutes may serve a critical function in the degradation of complex organic matter via interactions with other microbial phyla(Sun et al. 2021). This phenomenon reflects the functional synergy within microbial communities, which not only facilitates the decomposition of complex organic matter during composting but also establishes an ecological foundation for the succession of subsequent microbial taxa.

Further network analysis revealed that most key taxa exhibited relatively low relative abundances during the composting process. Despite their low relative abundances, these taxa play a critical role in maintaining the stability of the microbial community and regulating its functions(Liu et al. 2023). Our analysis revealed 17 key bacterial genera during the composting process, including representatives from the phyla Proteobacteria, Actinobacteria, Firmicutes, Gemmatimonadetes, and Chloroflexi. These taxa participate in material degradation and energy flow during composting via interactions with other microorganisms(Xun et al. 2021). These low-abundance yet functionally critical microbial taxa may represent key determinants of the stability of the composting system. These findings offer a novel theoretical basis for the optimization of composting processes.

This study elucidates the succession dynamics of bacterial communities during the composting of microbial inoculant-based compost and their associations with environmental factors. By integrating high-throughput sequencing and ecological network analysis, we further investigated the compositional shifts in microbial communities and their functional roles across distinct composting stages. This study demonstrated that the succession of microbial communities during composting is significantly influenced by environmental factors, including temperature, humidity, and pH. Additionally, the diversity and functional profiles of microbial communities distinctly shift across different composting stages(Wang et al. 2025). At the initial stage of composting, Firmicutes dominated the bacterial community. However, as composting progressed, taxa such as Proteobacteria and Bacteroidetes gradually became the dominant groups, reflecting the adaptability of the microbial community to environmental changes during composting. Ecological network analysis further elucidated the pivotal role of low-abundance yet functionally critical taxa throughout the composting process. Collectively, the results of this study provide a novel theoretical foundation for optimizing composting processes, enhancing composting efficiency, and improving the quality of compost products. Future research could further integrate multiomics technologies to elucidate the functional genes and metabolic pathways of microbial communities throughout the composting process, thereby enabling more refined optimization of composting processes.

6 Conclusion

In this study, an in-depth analysis of microbial community succession during the composting of microbial inoculant-based compost was conducted, revealing the dynamic patterns of bacterial diversity and community structure throughout the composting process. The findings of this study demonstrate that the microbial community undergoes marked succession throughout the composting process: thermophilic microorganisms dominate the initial stage, whereas as temperatures and environmental conditions shift in the later stage, other microbial groups gradually proliferate and become dominant. This process reflects the profound influence of environmental factors on the microbial community throughout composting, particularly in terms of functions such as organic matter degradation, nitrogen cycling, and carbon fixation, where the microbial community plays distinct functional roles across different stages.

In terms of the dynamic shifts in the microbial community, Firmicutes dominated during the early stage of composting; however, its relative abundance decreased significantly as composting progressed. Conversely, the relative abundance of bacterial groups such as Proteobacteria and Bacteroidetes gradually increased during the later stage, indicating that these taxa play critical roles in organic matter transformation and nutrient cycling during the later phase of composting. These shifts are closely associated with fluctuations in environmental factors—including temperature, pH, and moisture—throughout the composting process. Beta diversity analysis revealed significant dissimilarities among bacterial communities across different composting stages. Further PCoA (principal coordinate analysis) revealed distinct variations in the bacterial community composition among the early, middle, and late phases of composting, confirming the continuous succession of the microbial community structure throughout the composting process.

Furthermore, the community network analysis in this study revealed that the microbial community during composting exhibited small-world properties and a modular structure, with 17 key bacterial genera functioning as core players in the functional network of the microbial community. These genera are primarily affiliated with five phyla, namely, Proteobacteria, Firmicutes, and Bacteroidetes, and their interactions during composting contribute critically to the stability of the compost ecosystem. The stable occurrence of Ureibacillus thermophilus and the dynamic shift in the relative abundance of the genus *Ureibacillus*, characterized by an initial decrease followed by an increase, suggest that this taxon plays critical ecological roles during composting, particularly in organic matter degradation and temperature modulation.

Taken together, the results of this study not only provide novel insights into the dynamic shifts in microbial communities during composting but also offer a theoretical foundation for the optimization of composting processes. Further optimization of environmental control during composting—particularly the modulation of temperature and humidity—can facilitate the proliferation of key functional microorganisms, thereby increasing the efficiency and quality of the final compost product. Future research should focus on the expression of functional genes within microbial communities and explore

the interactions between microbial metabolic pathways and environmental factors during composting, with the aim of achieving more efficient composting technologies and resource recovery.

Declarations

CRediT authorship contribution statement

Yu Zhang: Conceptualization, methodology, investigation, formal analysis, data curation, writing–original draft, writing–review & editing, visualization, supervision. **Feng Huo:** methodology, formal analysis, data curation, writing–original draft, visualization, supervision. **Hong Xie:** Methodology, writing–review & editing. **Jie Zhang:** Data curation. **Hu Lou:** Methodology, writing–review & editing. **Guocai Zhang:** Conceptualization, methodology, writing–original draft, writing–review & editing, and funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics and consent to participate

Not applicable

Consent for publication

Not applicable

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Figures

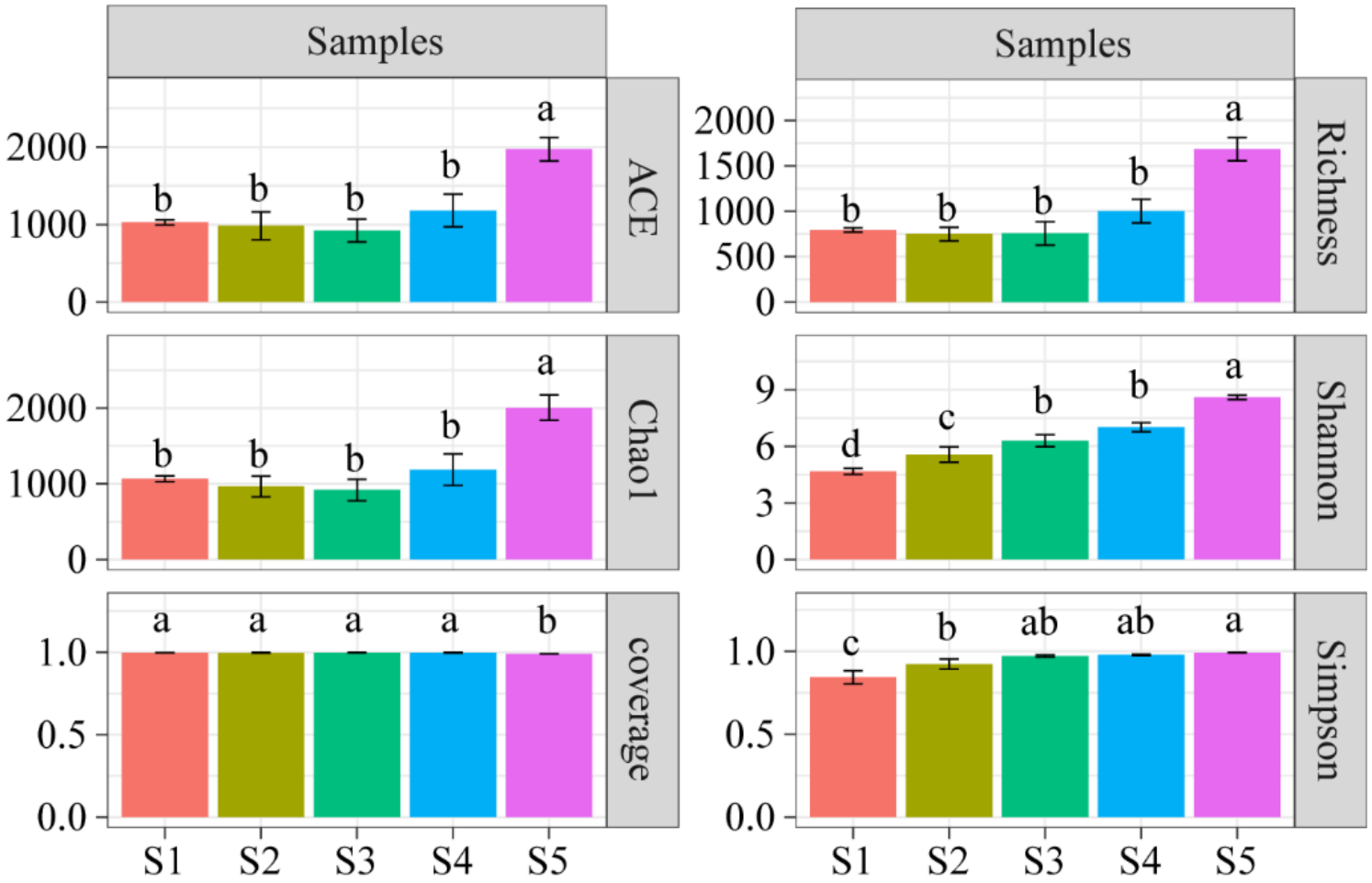


Figure 1

Alpha diversity indices for the 16S rRNA gene sequence in different samples

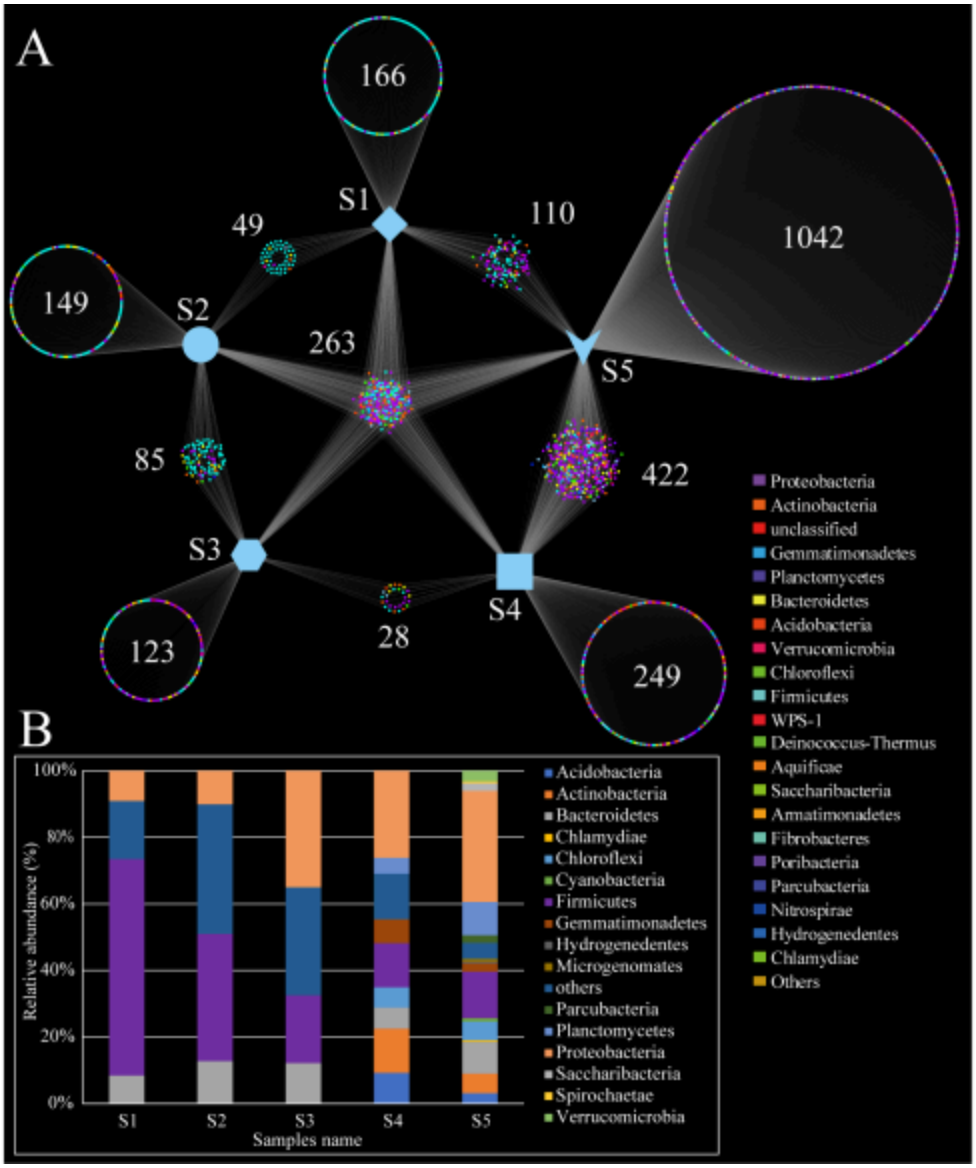


Figure 2

Venn diagram analysis showing the number of unique and shared OTUs in different

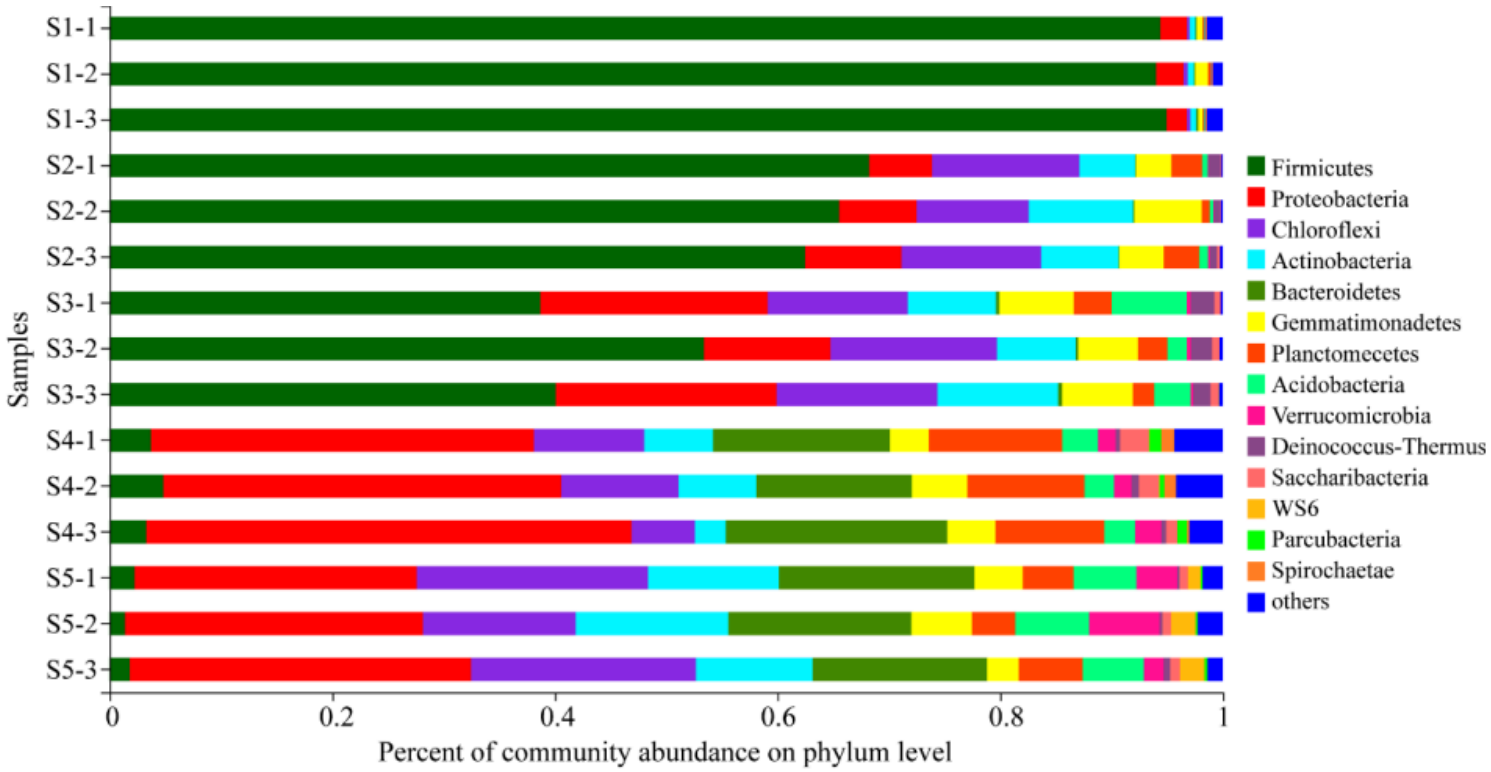


Figure 3

Relative abundance of bacteria at the phylum level in the different samples

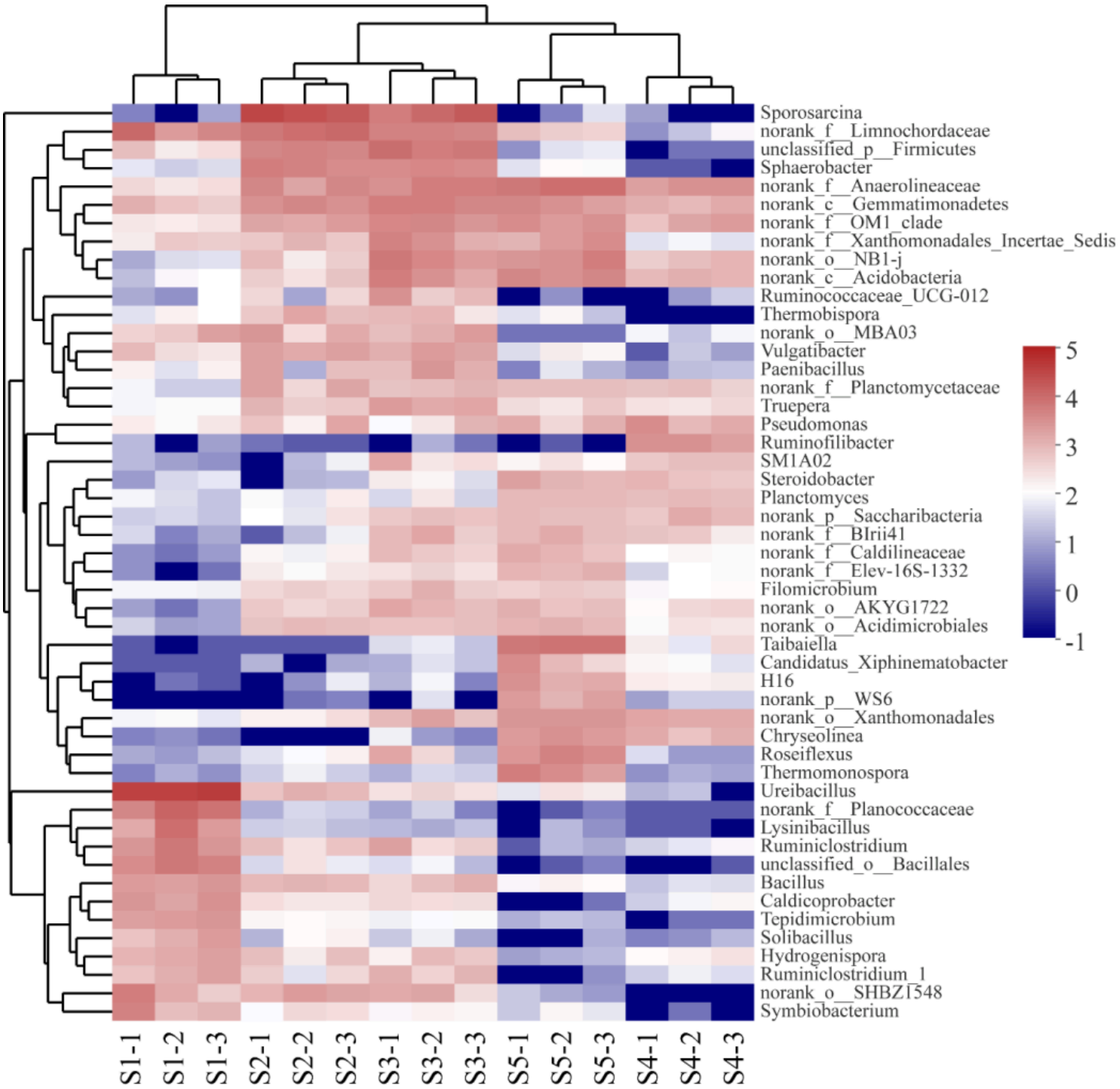


Figure 4

Heatmap of the bacterial distribution of the 50 most abundant genera in the different samples

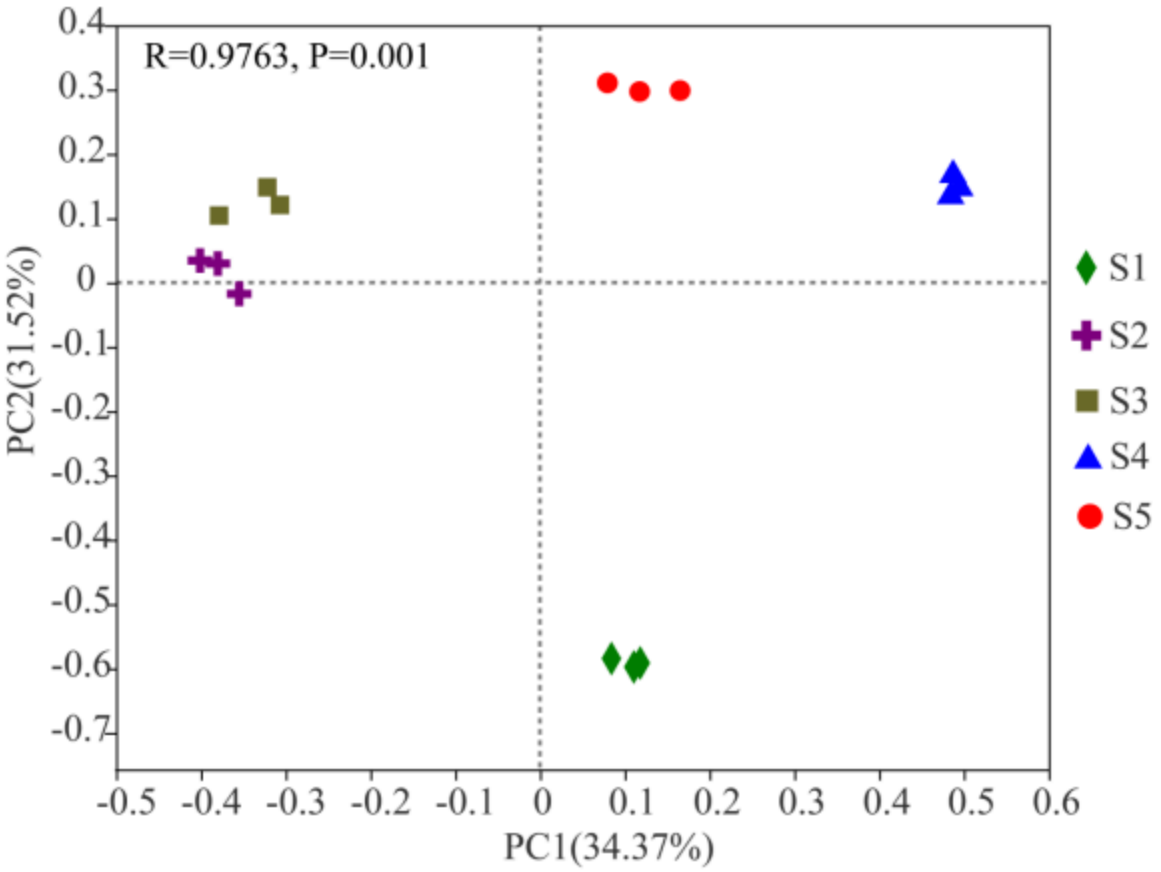


Figure 5

Principal coordinate analysis of the bacterial communities in different samples

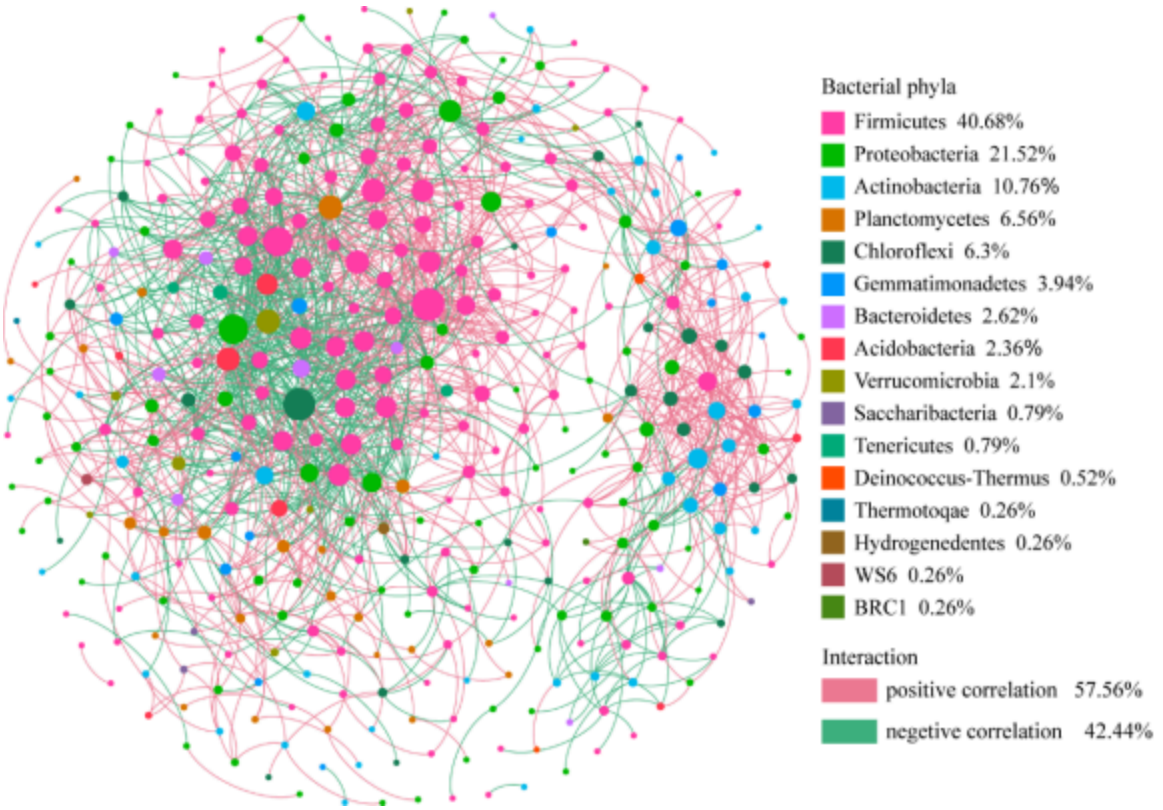


Figure 6

Phylogenetic molecular ecological networks

Note: A node represents an OTU, and its color represents its module. The green and red lines indicate negative and positive interactions, respectively. pp: positive interaction; np: negative interaction.

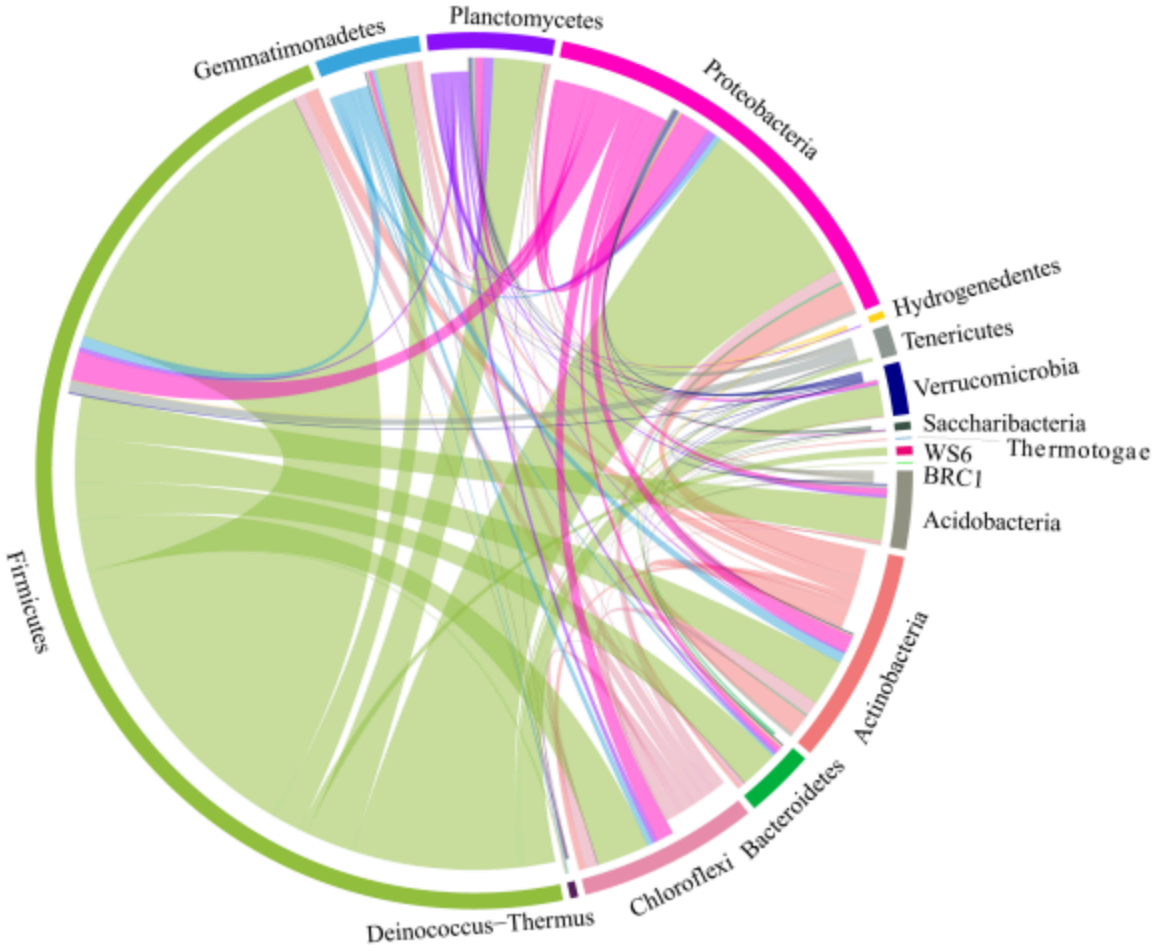


Figure 7

Circos plots showing the distribution of links among interacting phyla in different networks

Note: The band between two segments represents the network connections among the phyla, and the width indicates the number of connections.

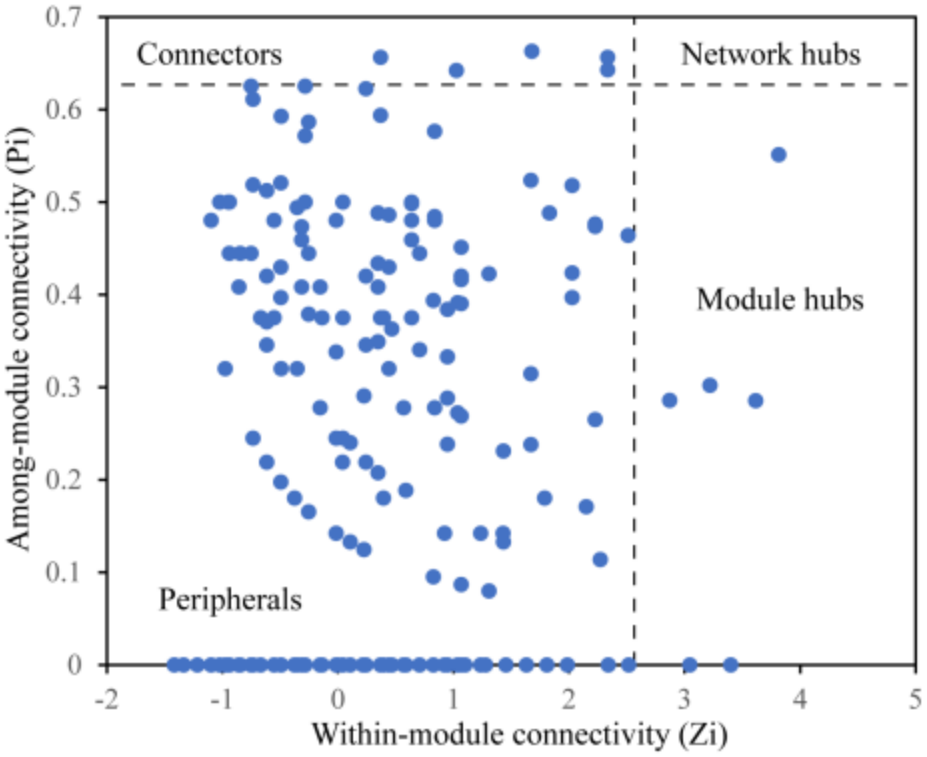


Figure 8

Putative keystone taxa in the different networks based on Pi and Zi

Note: According to the Pi and Zi values, the nodes are divided into peripherals, connectors, module hubs and network hubs with their possible ecological functions. The solid circle represents an OTU, and the color indicates different networks.

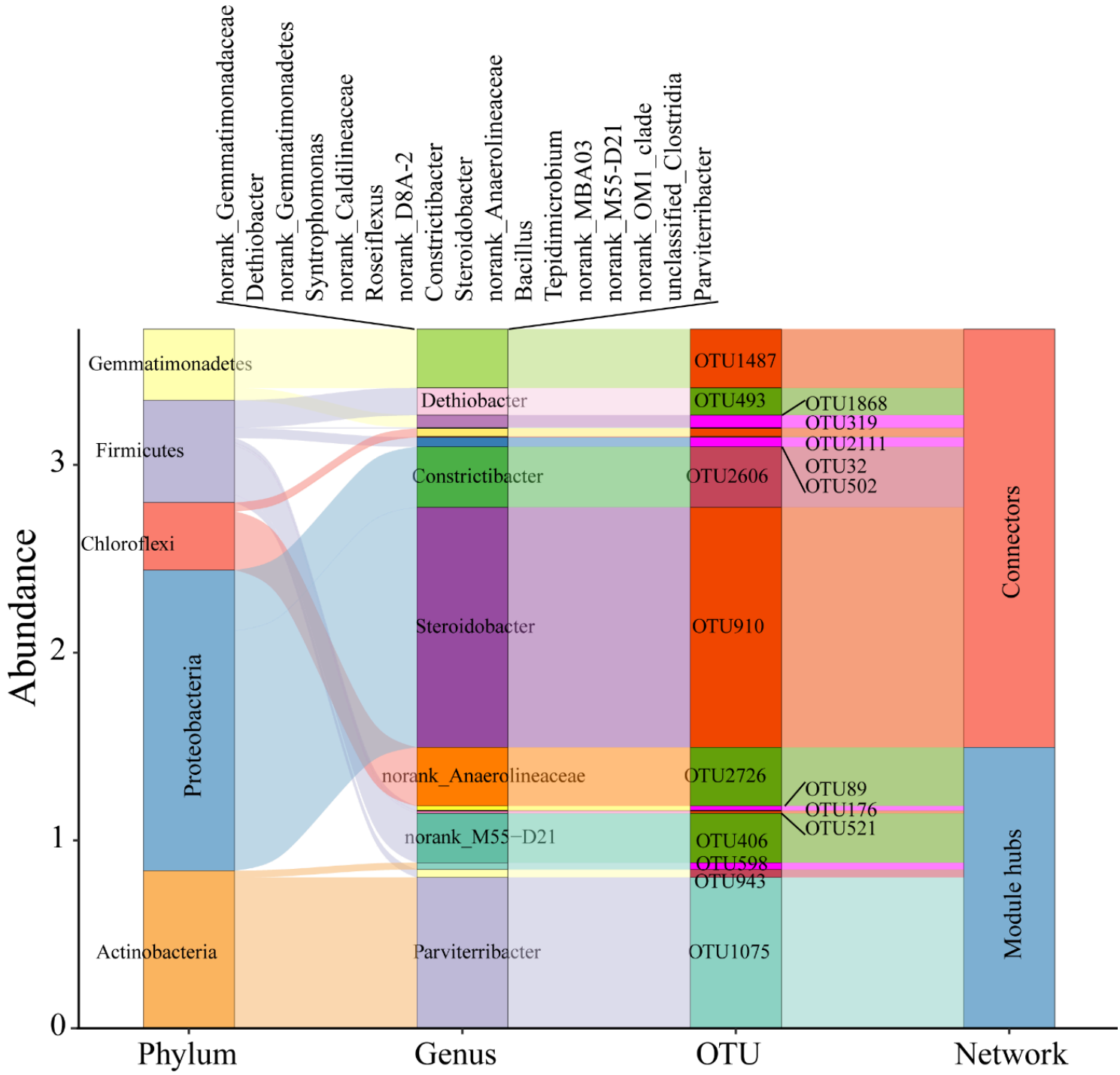


Figure 9

Putative keystone taxa and their system classification levels and relative abundance in different